OXYGEN CONSUMPTION OF WHOLE INSECTS AND INSECT HOMOGENATES [1]

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The alteration of enzyme activity by cell rupture has been extensively studied with vertebrate tissues, but very little work has been done with those of invertebrates. Bodine and Lu (1951) found that homogenization of both diapause and actively growing embryos of the grasshopper, *Melanoplus differentialis,* reduced oxygen consumption by approximately 65 per cent. Their results agree with the 60 to 70 per cent decrease obtained with vertebrate tissue (De Robertis, Nowinski and Saez, 1950). They concluded that 65 per cent of the oxygen uptake of intact embryos is due to structure and the remainder to the basic chemical composition of the cells.

In the present study, the oxygen consumption of homogenates of the Japanese beetle, *Popillia japonica,* and of the mealworm, *Tenebrio molitor,* was determined for each day of embryonic development at 30° C. A comparison was then made with the oxygen consumption of whole eggs. They lack a diapause and thus differ from the eggs of the grasshopper, *M. differentialis.* A similar comparison was made with whole insects and their homogenates in the larval stage, and for each day of the pupal stage of the mealworm at 30° C. Finally, a study was made of the effect of different homogenate concentrations on the oxygen consumption of larvae and pupae of the mealworm.

Material and Methods

Japanese beetle eggs were collected daily and placed on the surface of moist, firmly-packed soil in one-ounce metal salve boxes. Eggs of the mealworm were collected daily from beetles kept in white flour. They were stored in a desiccator over a saturated solution of NaCl (relative humidity, 75 per cent). Eggs of both species were incubated at 30° C.

Larvae of the mealworm, obtained from a mixed culture maintained at room temperature, were used in these experiments. Prepupae were collected from the same culture and placed at 30° C. They were examined daily and the newly molted pupae were placed in beakers and kept at 30° C. In this manner, pupae of known ages (within 24 hours) were obtained.

All homogenates were made in 0.03 molar phosphate buffer, adjusted to a pH of 7.4. The tissue was homogenized for one minute in a motor-driven glass homogenizer. This time was selected because no differences were found in the respiratory rates of tissues homogenized for one, three, or five minutes.

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Oxygen consumption was measured in Warburg constant volume manometers according to the procedure outlined by Umbreit, Burris and Stauffer (1945). The center well of the Warburg flask contained 0.2 ml. of a 10 per cent KOH solution to which was added a piece of folded filter paper to increase the surface of the KOH

FIGURE 1. Effect of homogenization on the O_2 consumption of insect eggs. Upper graphs, Japanese beetle eggs; lower graphs, mealworm eggs; solid lines, intact eggs; dotted lines, 10 per cent homogenates. Graph for intact Japanese beetle eggs taken from Ludwig and Wugmeister (1955).

TABLE I

Oxygen consumption of whole mealworm larvae and of homogenates of mealworm larvae

Per cent homogenate	No. of readings	Oxygen consumption μ liters/gram/minute (with standard errors)	Per cent decrease
Whole larvae	46	10.17 ± 0.73	
20	18	7.25 ± 0.28	28.7
10	71	6.62 ± 0.34	34.9
7.5	18	7.45 ± 0.73	26.7
5	42	6.06 ± 0.33	40.4
2.5	18	5.46 ± 0.58	46.3
1	49	3.65 ± 0.34	64.1

solution. The manometers were placed at a temperature of 30° ± 0.01° C. Readings were made at 15-minute intervals for a period of two hours. During this time the manometers were rocked horizontally 100 times per minute.

OBSERVATIONS

A comparison of the rates of oxygen consumption of whole Japanese beetle eggs and of their homogenates for each day of embryonic development is shown in Figure 1. Each point in this figure represents an average of at least 10 determinations. Respiration of 10 per cent homogenates, expressed as μ liters of oxygen/50 eggs/hour, was 5.1 in newly laid eggs. It increased to 26.2 during the next two days and remained relatively constant at approximately 30 during the remainder of the embryonic period. A comparison of these results with those of Ludwig and Wugmeister (1955) shows that homogenizing the newly laid egg reduced oxygen consumption by approximately 50 per cent. It did not change the respiratory rate from the third to the sixth days of incubation, but towards the end of the embryonic period, it again resulted in a reduction of more than 50 per cent. On the other hand, the amount of reduction was maintained constant throughout the embryonic period in the mealworm. Using 10 per cent homogenates, it was found that homogenizing the egg of this species resulted in a reduction of approximately 48 per cent in the rate of oxygen consumption (Fig. 1).

The rates of oxygen consumption of whole mealworm larvae and of their homogenates are listed in Table I. Oxygen consumption of whole larvae averaged 10.17 μ liters/gram/minute. That of homogenates depended upon concentration, being 7.25 in 20 per cent, and only 3.65 in 1 per cent homogenates. In the former case, the reduction amounted to approximately 29 per cent; and in the latter, to 64 per cent.

The effects of homogenization on the rate of oxygen consumption of the mealworm during metamorphosis are shown in Figure 2. Each point in Graph A represents an average of at least 10, in Graph B of 30, and in Graph C of 12 determinations. The oxygen consumption of whole insects followed the characteristic U-shaped curve first described for this species by Krogh (1914). It was high immediately after pupation, decreased to a minimum in the two-day pupa, and then increased during the remainder of the pupal stage. The oxygen uptake of 1 per cent

and of 5 per cent homogenates remained relatively constant during the pupal stage, but increased in newly emerged adults. Hence, the amount of reduction resulting from homogenization varied with the stage of metamorphosis. With 1 per cent homogenates, it was approximately 61 per cent in prepupae, 80 per cent in newly molted, 50 per cent in two-day, 72 per cent in five-day pupae, and 80 per cent in newly emerged adults. The oxygen uptake of 5 per cent homogenates was considerably higher than that of the 1 per cent. However, its rate was also relatively constant throughout the pupal stage.

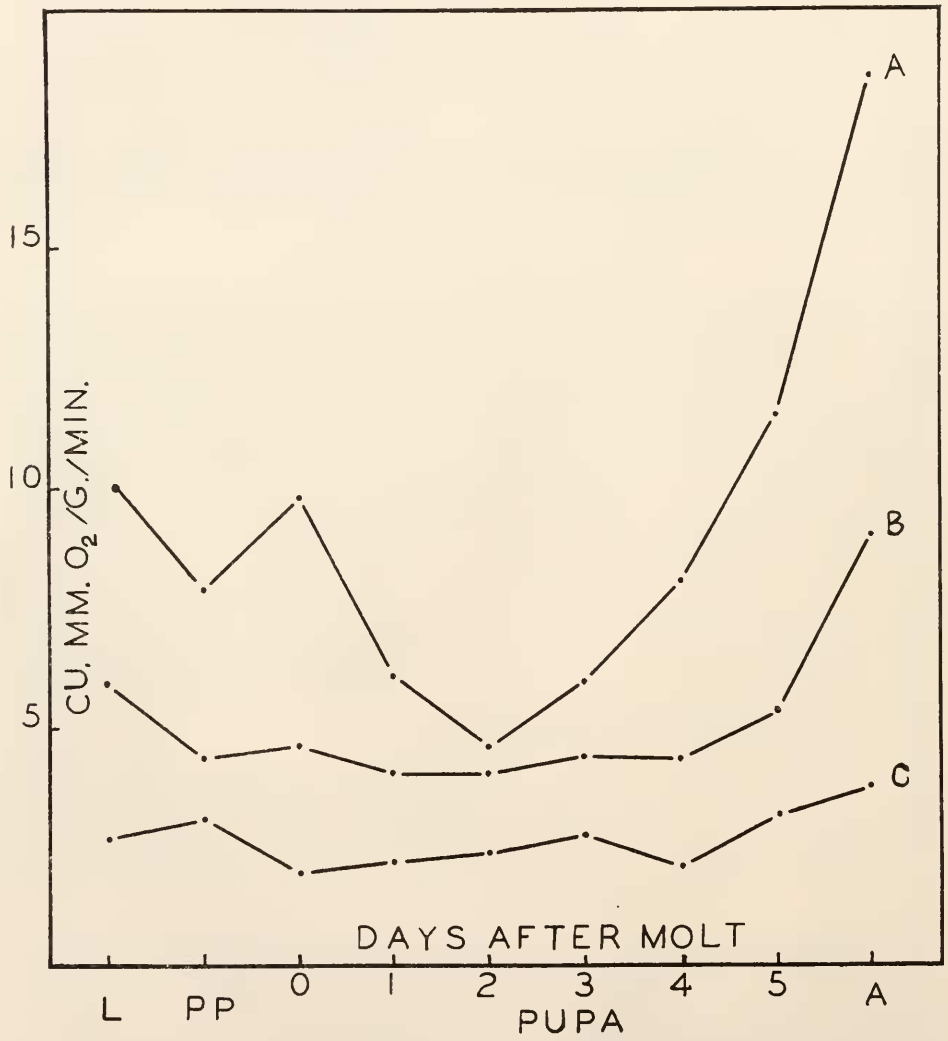

FIGURE 2. Effect of homogenization on the O_2 consumption of mealworms during metamorphosis. Graph A, intact insects; graph B, 5 per cent homogenates; graph C, 1 per cent homogenates; L, larvae; PP, prepupae; A, newly emerged adults.

Discussion

The varying effects of homogenization on oxygen consumption throughout the embryonic period of the Japanese beetle may possibly be correlated with water content and degree of organization of the egg. Rothstein (1952) reported that the newly laid egg weighed only 0.83 mg. and contained 50 per cent water. Hence, the various constituents are in a rather concentrated condition. During the first four days of development at 30° C., the egg increased in weight to 2.3 mg. and its water content increased to more than 80 per cent. Hence, approximately 1.5 mg. of water were imbibed by each egg and the enzymes and other constituents were greatly diluted. At this time homogenization appears to have no effect on the rate of oxygen consumption. Gese (1953) found that blastokinesis occurs on the fifth day at 30° C., followed by the differentiation of the embryo. This period of differentiation was shown by Ludwig and Wugmeister (1955) to be associated with a rapid increase in respiratory rate and an increase in the activities of cytochrome oxidase and succinic dehydrogenase. Homogenization of the egg during this period of differentiation resulted in a marked reduction in oxygen consumption, probably due to the disruption of organization in the egg. On the other hand, mealworm eggs do not imbibe water but maintain a constant weight throughout the embryonic period. Homogenization of these eggs resulted in a constant reduction of approximately 48 per cent in oxygen consumption. The difference between the present results with mealworm eggs, and those of Bodine and Lu (1951) with grasshopper eggs (48 as compared to 65 per cent), may be associated with homogenate concentration. Furthermore, Bodine and Lu used embryos only, while in the present experiments whole eggs were used.

The elimination of the U-shaped respiratory curve in the pupal stage of an insect by homogenization was also reported by Cotty (1955) with the house fly, *Musca domestica*. These observations add evidence for the belief that the U-shaped curve is associated with different degrees of tissue organization (Krogh, 1914; Fink, 1925). When this organization is disrupted by homogenization, a uniform rate is produced. However, homogenization may also affect respiratory enzymes and thus modify the rate of oxygen consumption as indicated by the work of Handler and Klein (1942) who showed that DPN is rapidly broken down by tissue homogenates thus blocking various dehydrogenases.

The present experiments indicate that the effects of homogenization on the respiratory rate of insect tissues vary with the stage of development, physiological differences among various species, and homogenate concentration.

Summary

1. The oxygen consumption of 10 per cent homogenates of Japanese beetle and mealworm eggs was measured for each day of the embryonic period at 30° C. and compared with that of intact eggs.

2. Homogenization of newly laid Japanese beetle eggs resulted in a reduction of 50 per cent in oxygen consumption. From the third to sixth day, there was no decrease but towards the end of the embryonic period, a reduction of more than 50 per cent was again obtained. Homogenization of mealworm eggs resulted in an average reduction of 48 per cent in oxygen consumption which remained constant throughout the embryonic period.

3. The rates of oxygen consumption of whole mealworm larvae and of their homogenates were determined. Homogenization resulted in a decrease in respiratory rate, the extent depending on homogenate concentration. With 20 per cent homogenates, there was a reduction of 29 per cent; and with 1 per cent homogenates, it was 64 per cent.

4. The effects of homogenization on the respiratory rate of mealworms were determined for each day of metamorphosis at 30° C. Homogenization abolished the U-shaped respiratory curve characteristic of intact pupae. Therefore, the degree of reduction depended on the stage of metamorphosis. With 1 per cent homogenates, it was 61 per cent in prepupae, 80 per cent in newly molted, 50 per cent in two-day, 72 per cent in five-day pupae, and 80 per cent in newly emerged adults. Furthermore, the extent of reduction also varied with homogenate concentration being greater in 1 per cent than in 5 per cent homogenates.

LITERATURE CITED

Bodine, J. H., and K. H. Lu, 1951. Structure and endogenous oxygen uptake of embryonic cells. *Physiol. Zool.*, **24**: 120–126.

Cotty, V. F., 1955. Respiratory metabolism of prepupae and pupae of the house fly, *Musca domestica* L., and of their homogenates. Ph.D. thesis, New York University.

DeRobertis, E. D. P., W. W. Nowinski and J. F. Saez, 1950. General cytology. W. B. Saunders Co., Philadelphia.

Fink, D. E., 1925. Metabolism during embryonic and metamorphic development of insects. *J. Gen. Physiol.*, **7**: 527–543.

Gese, E. C., 1953. Embryogenesis of the Japanese beetle, *Popillia japonica* Newman, and a correlation of morphological events with known physiological changes. Ph.D. thesis, New York University.

Handler, P., and J. R. Klein, 1942. The inactivation of pyridine nucleotides by animal tissues *in vitro*. *J. Biol. Chem.*, **143**: 49–57.

Krogh, A., 1914. On the rate of development and CO_2 production of chrysalides of *Tenebrio molitor* at different temperatures. *Zeitschr. f. allg. Physiol.*, **16**: 178–190.

Ludwig, D., and M. Wugmeister, 1955. Respiratory metabolism and the activities of cytochrome oxidase and succinic dehydrogenase during the embryonic development of the Japanese beetle, *Popillia japonica* Newman. *J. Cell. Comp. Physiol.*, **45**: 157–165.

Rothstein, F., 1952. Biochemical changes during the embryonic development of the Japanese beetle (*Popillia japonica* Newman). *Physiol. Zool.*, **25**: 171–178.

Umbreit, W. W., R. H. Burris and J. F. Stauffer, 1945. Manometric techniques and related methods for the study of tissue metabolism. Burgess Publishing Co., Minneapolis.